2009 Gypsy Moth Suppression

Project Summary and Results

Monongahela National Forest, West Virginia

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ABSTRACT

INTRODUCTION

Aerial surveys conducted by the USDA Forest Service and the West Virginia Department of Agriculture identified 8,585 acres of oak forest defoliated by the gypsy moth this year on the Monongahela National Forest (MNF). This is a large decline from the 27,866, 46,039, and 42,515 acres defoliated in 2002, 2001, and 2000, respectively. Many of the areas defoliated this year were also defoliated in 2002, 2001, and 2000.

GYPSY MOTH

The gypsy moth, *Lymantria dispar* (Linneaus) is a non-native defoliator of forest, shade and ornamental trees throughout the Northeastern United States. Since its intentional importation and accidental release in eastern Massachusetts in 1869, the gypsy moth has steadily expanded its range. Despite many attempts to halt its spread westward from the northeastern United States, West Virginia experienced its first gypsy moth defoliation in 1985. Since that time, the gypsy moth has defoliated nearly 2.4 million acres in the state.

The gypsy moth produces one generation per year. Larvae begin hatching from egg masses in late April and early May when tree buds begin to open. At this time, larvae go through an obligatory dispersal period where they leave the vicinity of the egg, moving upward and spinning a thread of silk as they go (Leonard 1981). Eventually the wind catches the larvae and disperses them. Airborne larvae are carried and deposited some distance downwind from the source with the following results: 1) larvae will land on or crawl onto acceptable host plants and begin feeding; 2) larvae will land on either acceptable or unacceptable host plants and re-disperse; 3) larvae will be deposited into areas unacceptable for survival and re-dispersal where they will die (Mason and McManus 1981). The larvae feed for two to three months completing their development by late June and early July and seek sheltered areas in which to pupate. The pupal period last anywhere from 10 days to two weeks. After emerging from the pupal case the females, which cannot fly, crawl a short distance and emit a pheromone scent to attract males. After mating, the female lays a single egg mass that contains from 75 to 1,000 eggs, which she covers with hairs from her abdomen giving it a fuzzy brown texture and color. The egg masses over winter and hatch the following spring.

The number of host trees and shrubs fed on by the gypsy moth exceeds 300 species, with species of oaks (*Quercus* spp.) ranked among the most favored (Leonard 1981). Gypsy moth is an outbreak species whose populations can remain at low levels for several years, then undergo large population increases in a matter of one or two years. After populations have increased to an outbreak density they can remain high for one to five years, outbreaks decline suddenly to low densities where it is difficult to find any life stage (Liebhold et al. 2000). The main effects of gypsy moth feeding on individual trees involves the depletion of root carbohydrate food resources leading to a reduction in growth, reproduction, and increased vulnerability to secondary agents of mortality. Heavy defoliation forces re-foliation which occurs when about 60 percent of the foliage is lost (Liebhold et al. 1994). This re-foliation uses carbohydrate reserves in trees and can increase their vulnerability to drought and to other insects and diseases. This defoliation and subsequent tree mortality can alter wildlife habitats, change water quality and

temperature, increase forest floor temperatures and light levels and reduces aesthetic, recreational, and property values of forests and urban environments.

Bacillus thuringiensis

The only biological insecticide currently registered and commercially available for gypsy moth control is the microbial insecticide, Btk. This microbial insecticide is available through two manufacturers and contains both the spore and crystal as the entomopathogenic ingredients (Reardon et al. 1994). Btk has been used extensively in suppression projects throughout the U.S. in both forested and residential areas. Btk is a bacterium that acts specifically against lepidopterous larvae as a stomach poison and therefore must be ingested. The major mode of action is by midgut paralysis, which occurs soon after feeding. This results in a cessation of feeding and death by starvation. Btk has been shown to impact other non-target caterpillars that are exposed to the treatment and are actively feeding. Btk is persistent on foliage for about 7 to 10 days.

Gypchek®

A second microbial insecticide that is registered and available in limited quantities is the formulated nucleopolyhedrosis virus called Gypchek® (U.S. Forest Service, USDA, Washington, DC). This product is not available commercially but is produced in limited quantities by a cooperative effort of the USDA Forest Service and the Animal Plant Health Inspection Service (APHIS). The active ingredient in Gypchek formulations has a very narrow host range (lymantriids) and occurs naturally in gypsy moth populations. Normally the virus reaches epizootic proportions when gypsy moth populations reach high densities because of increased transmission within and between gypsy moth generations. The application of Gypchek to gypsy moth populations simply expedites this process by increasing the exposure of the virus at an earlier stage. Healthy, feeding gypsy moth caterpillars become infected by ingesting contaminated foliage and soon stop feeding and die.

AIRCRAFT AND SPRAY EQUIPMENT

Contractor:	Summit Helicopters
Aircraft:	Bell 205, Bell 206L4, Bell 206L3
FAA Number:	N2773H, N6344D, N5744Y
Pilot:	Tom Hanks, Dexter Williams, Ron Jackson, Jim Carolton
DGPS Brand:	AG-NAV
Nozzle Type:	Micronair AU5000
Air Speed:	70 to 90 mph, depend on the aircraft type
Swath Width:	100 feet.
Dates Sprayed:	May 13 thru 22
Insecticide:	Single and Double application of Foray 76 B @ 25 BIUs/ac @ 43 ounces/ac, double applications of Gypchek® applied at $2x10^{11}$ occlusion bodies (OB's)/acre at the application volume of 0.5 gal/ac.

PRE-TREATMENT SAMPLING, SPRAY COVERAGE, AND CONDITIONS

Timing of the treatment application is generally dictated by insect and foliage development. For this reason, the USDA Forest Service has established certain guidelines (United States Department of Agriculture 2001) for the application of GypchekBtk products (Tables 1 and 2). These guidelines are the result of research and the consensus of experts in the field of aerial application.

Table 1. Pre-Treatment Larval and Foliage Development Guidelines for Btk product treatment of Lymantria dispar.

Gypsy Moth	Spray when 80 percent of the larvae are in the 1st instar and 20 percent are in the 2nd instar
Oak Foliage	Expanded 30 percent or more

Table 2. Pre-Treatment Weather Guidelines for Btk product treatment of Lymantria dispar.

Wind Velocity	Wind velocity must be 10 mph or less when measured in or near the spray block with a hand-held wind gauge.
Probability of Precipitation	Probability of precipitation within 6 hours after the completion of spraying must be 50 percent or less.
Air Temperature	Air temperature in the shade at approximately 5 feet above the ground must be 40° F to 80° F.
Wet Foliage	Foliage must not be dripping wet either from precipitation or overnight dew.
Relative Humidity	Relative humidity must be 50 percent or greater.

Beginning on April 17, the Lake Sherwood spray block was visited every 7 to 11 days to measure gypsy moth emergence, larval dispersion, and leaf development (Table 3). The western block was deemed ready for spraying on May 16, and spraying commenced at 1315 and terminated at 1400 because of weather-related issues (Appendix A). The block was finished on May 20. Spraying on the eastern block commenced on May 20 at 1600 and terminated at 1800 because of darkness. The block was finished on May 24 from 1045 to 1300. Weather conditions (Table 4) were good during application, but application had to be delayed several times because of fog and rain concerns.

Table 3. Larval and foliage development at Lake Sherwood, Monongahela National Forest, for the 2003 suppression project for Lymantria dispar.

Date	Larval Emergence	Foliage Development	Larval Development
4/17	none	white oak buds (closed)	none
4/28	27% emergence	white oak buds (closed-swelling)	100% first instar

5/5	16% emergence	white oak buds (swelling to <20%)	100% first instar
5/12	25% emergence	white oak 20%	49% first instar 50% second instar 1% third instar
5/16*	-	white oak 20-50%	31% first instar 59% second instar 9% third instar
5/20*	-	white oak 20-50%	22% first instar 77% second instar 1% third instar
5/24*	-	white oak 20-50%	5% first instar 75% second instar 20% third instar

* Treatment dates

Table 4. Meteorological conditions at Lake Sherwood, Monongahela National Forest, during the 2003 suppression project for Lymantria dispar.

Date	Time	Temperature Range (^{0}F)	Relative Humidity Range (%)	Wind Speed Range (mph)
5/16	1300 - 1415	63 - 75	33 - 89	0 - 4
5/20	1630 - 1830	62 - 71	46 - 90	0 - 5
5/24	1030 - 1315	56 - 65	56 - 83	0 - 3

Spray Coverage

Based on observations made by personnel in the treatment block, enzyme-linked immunosorbant assay (ELISA) tests (Table 5), and viewing of the AG-NAV printout (Appendix B), spray coverage throughout the spray block was excellent. No skips occurred within the spray block, and very little area outside the treatment block was sprayed.

Table 5. Enzyme-linked immunosorbant assay (ELISA) tests for Bacillus thuringiensis var. kurstaki (*Btk) proteins on foliage at Lake Sherwood, Monongahela National Forest, 2003 suppression spray blocks.*

Date	Location	Sample	Results	Sample	Results	Sample	Results	Sample	Results
5/19	FS# 311 top	A1	20-100*	A2	20-100	A3	20-100	A4	20-100
	FS 311 bottom	B1	>100	B2	>100	B3	>100	B4	>100
	Negative control	N1	0	N2	0	-	-	-	-
	Positive control	P1	20	P2	20	-	-	-	-
5/20	FS 55 top	A1	20-100	A2	20-100	A3	20-100	A4	20-100
	FS 55 mid	B1	20-100	B2	20-100	B3	20-100	B4	20-100
	Negative control	N1	0	N2	0	-	-	-	-
	Positive control	P1	20	P2	20	-	-	-	-
5/24	FS 815 top	A1	20-100	A2	20-100	A3	20-100	A4	20-100
	FS 815 mid	B1	20-100	B2	20-100	B3	20-100	B4	20-100

Negative control	N1	0	N2	0	-	-	-	-
Positive control	P1	20	P2	20	-	-	-	-

[#] Location within spray block along Forest Service Road (FS)

* ng/Btk proteins/ml (designations: less than 20 = "low", 20-100 = "moderate", greater than 100 = "high")

POST-TREATMENT SAMPLING AND CONDITIONS

July and September aerial and ground detection surveys (Table 6) found no gypsy moth defoliation or new egg masses within the spray blocks. Because of the cool, wet spring, oak anthracnose was present and affecting trees throughout the spray blocks.

Table 6. Lymantria dispar population reduction results for the Lake Sherwood, Monongahela National Forest, 2003 suppression project.

Block	No. Acre	Pre-Trt. Defoliation[#]	Post-Trt. Defoliation[#]	Pre-Trt (EM/AC*)	Post-trt (EM/AC*)	Treatment Threshold	Insecticide	No. Applic.
1	1967.5	Heavy[#]	light	12,869	0.0	>1000	Foray 76B	2
2	598.0	Light	light	6389.3	0.0	501-1000	Foray 76B	2
3	1425.1	Light	light	4880.8	0.0	501-1000	Foray 76B	2
4	4219.7	Light	light	1704.7	0.0	251-500	Foray 76B	1
5	1482.2	Light	light	4460.1	0.0	251-500	Foray 76B	2
6	160.2	Light	light	2420.3	0.0	251-500	Foray 76B	1
7	445.8	Light	none	1505.5	0.0	251-500	Foray 76B	1
8	1971.5	Light	light	5749.7	0.0	251-500	Foray 76B	2
9	397.6	Light	light	74.3	0.0	251-500	Foray 76B	1
10	83.2	Light	light	1693.3	0.0	>1000	Gypchek®	2
11	189.6	Heavy	Heavy	11770.5	0.0	501-1000	Gypchek®	2
12	198.2	Light	Heavy	5262.8	0.0	>1000	Gypchek®	2

*EM/AC = Egg masses per acre

[#] Based on aerial and ground detection surveys

Entomophaga maimaiga

The fungal pathogen, *Entomophaga maimaiga* (Humber, Shimazu, and Soper), is likely an introduced species. Although the exact origin of its introduction is a mystery and open to debate (see Hajek et al. 1995 for a history), its first widespread dramatic appearance in the Northeastern United States in 1989 (Hajek et al. 1990? This isn't in the ref. list) confirmed that this virulent and fast-spreading fungus had become established (Hajek et al. 1999? This isn't in the ref. list).

The life cycle of *E. maimaiga* appears to be closely synchronized with that of the gypsy moth and other spring defoliators. The fungus persists over winter as a resistant and dormant azygospore or resting spore in the forest litter, soil, or on the bark of trees (Weseloh 1999, Hajek and Humber 1997). According to Reardon and Hajek (1998), these spores have an obligate dormant period after production and asynchronously germinate throughout springtime (i.e. germinate to infect early through late stage GM larvae). In the spring, the resting spores produce germ conidia, which are released into the air and, if intersected by gypsy moth larvae, germinate on the larva's integument to begin an infection (Weseloh 1999). Mechanical pressure and enzymatic degradation (Hajek 1999) allow the fungus to penetrate the larva's body. High humidity is necessary for conidial production and discharge, and free water is required for

conidial germination (Reardon and Hajek 1998). Maximum infection in larvae exposed to resting spores in the soil usually occurs 1 to 2 days after significant precipitation (Weseloh and Andreadis 1992? This isn't in the ref. list). The fungus develops within the larva's body and death occurs within 6 to 9 days or less (Weseloh and Andreadis 2002, Hajek 1999). Early instar cadavers produce conidia almost exclusively, while older cadavers usually bear germ spores but can discharge conidia (Hajek 1999). The externally produced conidia produced at this time can infect other larvae. This process can be repeated as long as temperature and humidity are favorable. *E. maimaiga* is only known as a larval pathogen. In general, cadavers of early instars killed by the fungus are found on the foliage; later instar cadavers frequently remain attached to the lower tree trunk by their prolegs in a head down position (Reardon and Hajek 1998). They have a rubbery texture and appear dry. Some larvae will fall from the tree in 9 to 10 days, while some will remain attached throughout the autumn and winter. As the season progresses, the internally produced resting spores from late instar cadavers are eventually leached back into the soil, where they remain in a dormant state through the fall and winter (Reardon and Hajek 1998).

Weseloh (1999) suggested that the abundance of resting spores in the soil might have a large effect on the impact the fungus has on the gypsy moth. In order to assess the presence of spores and quantify the effect of the fungus on developing larvae within the Lake Sherwood spray blocks, soil samples and larvae collections were made throughout the spring. Soil samples were collected from three plots along Forest Service Road 311 (Figure 1) using the soil collection methods and direct soil bioassay developed by Weseloh and Andreadis (2002). Gypsy moth larvae were also collected from these sites to assess the infection rate in developing larvae.

Collection of soil samples

Forest soil samples were collected on April 17 directly next to the trunk of three oak [*Quercus* spp.] trees in each plot. These samples were taken by brushing away the litter layer and removing a 10-cm square area of soil about 3 to 6 cm from each of the four cardinal directions around the base of the tree. All sub-samples from a single tree were pooled and mixed. Samples were taken back to the laboratory and refrigerated at 4^{o} C.

Laboratory direct soil bioassay

Sub-samples of 40 ml were taken from each soil sample and spread over five water-saturated filter papers (90 mm) placed in the bottom of a plastic petri dish (100 X 15 mm). The soil was kept saturated with distilled water and held at 17^{0} C and 15 hours of light/day. On April 24, 1 week after establishment, a 90-mm sterilized circle of aluminum window screen was placed on top of the soil and 12 laboratory reared gypsy moth larvae [2nd or 3rd instars, provided by the ARS Beneficial Insect Research Laboratory (BIRL), Newark, Delaware] were placed on top of the screen. After 24 hours, the larvae were removed and reared individually in 40-ml plastic cups filled one-quarter full with gypsy moth diet [provided by BIRL] to determine fungal infection. Larvae were held until May 5 and examined periodically for fungal infection. Any dead larvae were dissected and examined under a compound microscope (magnification X400) to determine cause of death.

Field-collected gypsy moth larvae samples

At each site, 25 GM larvae were collected from egg masses, tree trunks, branches, and the foliage of plants (that could be reached from the ground) three times between May and June. Burlap bands were placed around five oak trees at each plot in early June to help collect late instar larvae. The field-collected larvae were reared individually in 40-ml plastic cups filled one-quarter full with gypsy moth diet [provided by BIRL] to determine fungal infection. Larvae were held for 14 days at 17^0 C and 15 hours of light/day and examined periodically for fungal infection. Any dead larvae were dissected and examined under a compound microscope (magnification X400) to determine cause of death.

Results

Based on results from the bioassay, the *Entomophaga maimaiga* resting spore infection rate ranged from 5 to 41 percent (Table 7). Field-collected 1st and 2nd instar GM larvae had *E. maimaiga* mortality rates of 8 to 20 percent, and 3rd and 4th instar GM larvae had mortality rates of 64 to 83 percent (Table 8). No 5th or later instar GM larvae were found at any sample plots within the spray blocks.

Table 7. Percent infection of Lymantria dispar (GM) larvae with Entomophaga maimaiga (EM) in laboratory direct soil bioassays of forest soil collected April 14 2003 at Lake Sherwood, Monongahela National Forest.

Location	Plot	Sub-sample	No. GM Alive	No. GM Dead	No. GM w/EM	Percent Infection
Control[#]	NA	1	12	0	0	0
	NA	2	13	1	0	
	NA	3	13	0	0	
FS 311(top)*	1	1	11	1	1	5.7
	1	2	11	0	0	
	1	3	11	1	1	
FS 311(middle)	2	1	6	3	3	26.5
	2	2	10	3	3	
	2	3	9	3	3	
FS 311 (bottom)	3	1	10	2	2	41
	3	2	3	9	7	
	3	3	10	5	5	

[#]Sterilized soil used for control *Location along Forest Service Road 311

Table 8. Percent infection of Lymantria dispar (GM) larvae with Entomophaga maimaiga (EM) in field-collected larvae May 15 and June 9, 2003 at Lake Sherwood, Monongahela National Forest.

Collection date		May 15, 2003				June 9, 2003			
Location	Plot	Instar	*n*	No. GM w/EM	Percent Infection	Instar	*n*	No. GM w/EM	Percent Infection
FS 311 (top)	1	1 and 2	26	2	8	3 and 4	14	9	64
FS 311 (middle)	2	1 and 2	34	5	15	3 and 4	30	25	83
FS 311 (bottom)	3	1 and 2	25	5	20	3 and 4	31	21	68

* Location along Forest Service Road 311

DISCUSSION AND CONCLUSIONS

Evaluating gypsy moth suppression project treatment effectiveness requires taking into account several different factors relating to the application of the pesticide and the effects of "natural" control factors such as *E. maimaiga* and the nuclear polyhedral virus (NPV). The decision to treat or allow natural factors to intervene is currently difficult to assess because we cannot predict weather conditions and the population dynamics of gypsy moth until after the treatment has been applied and this requires an intensive sampling regime to collect the data required to make these predictions. For this project, we do know that the flight files from the aircraft DGPS system and ELISA tests of the foliage, and the good environmental conditions post-treatment support that the pesticide was applied properly and in the amounts necessary to be conducive to good control. Aerial and post-treatment egg mass surveys showed little visible defoliation and no egg masses within the treatment areas. Soil and field assays of GM documented that *E. maimaiga* caused dramatic mortality of late instar larvae within the sample plots. The localized high populations of GM and the wet spring and summer during this year were "ideal" for *E. maimaiga* germination and continued conidia production throughout the developmental period of the GM. Future research is needed for land managers to be able to predict the impact of "natural" control factors, such as *E. maimaiga*, to make a "treat" or "no treat" decision when National Forest resource values, such as recreation, timber, and wildlife, would be at risk from potentially damaging gypsy moth populations.

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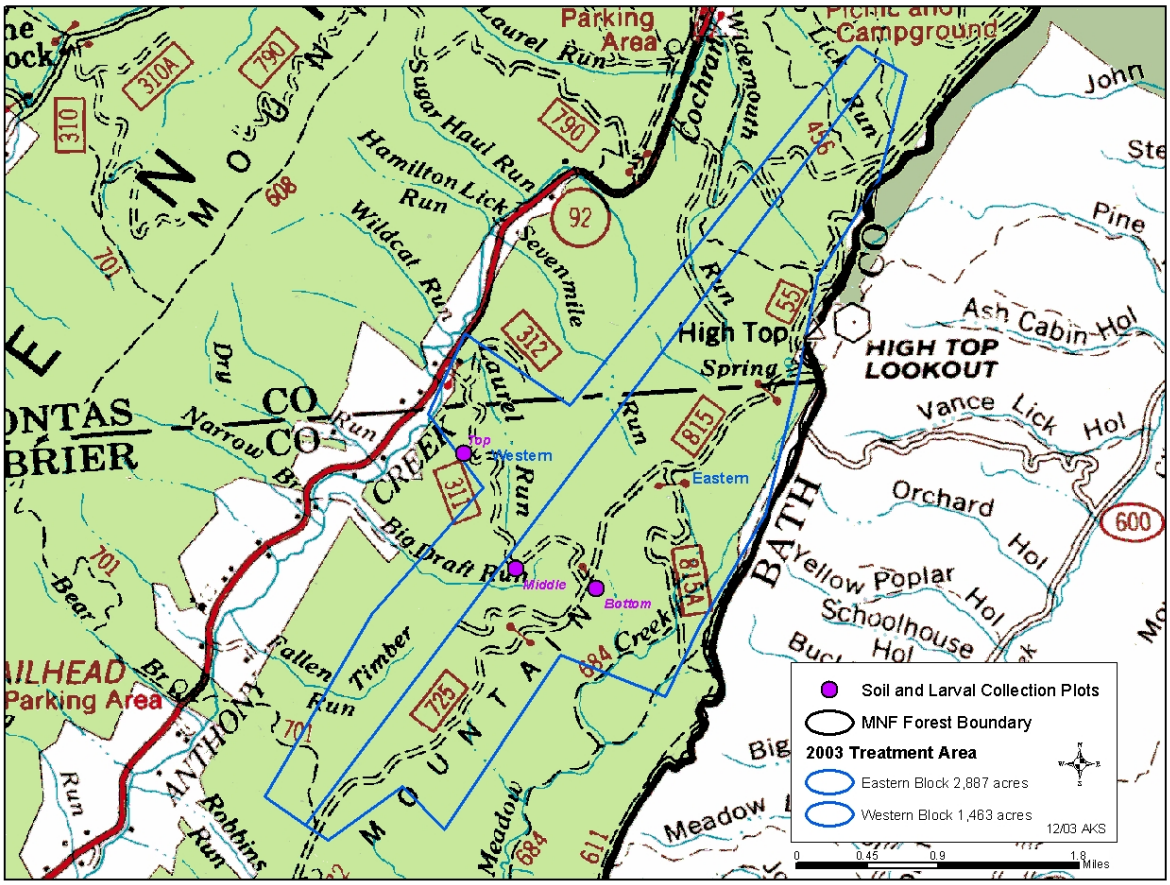
Figure 1. MNF 2003 Gypsy Moth Treatment Area.



Appendix A

Daily Rainfall Amounts After Treatment

Date	Rainfall Amount (inches)
5/16*	0.10
5/17	0.43
5/18	0.63
5/19	0.01
5/20*	0.00
5/21	0.08
5/22	0.04
5/23	0.70
5/24*	0.04
5/25	0.00
5/26	0.11
5/27	0.11
5/28	0.06
5/29	0.01

* Treatment dates

Appendix B

AG-NAV Map